THE DEVELOPMENT OF THE ACID PHOSPHATASE TEST FOR PROSTATIC CARCINOMA

*The Sixth Ferdinand C. Valentine Memorial Lecture**

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It is an honor indeed to have your Section on Urology take notice of certain studies made by my late wife, Ethel Benedict Gutman, and myself at the Columbia-Presbyterian Medical Center about 30 years ago. To acknowledge your generous Ferdinand C. Valentine Award for these studies I have been advised that it would be appropriate to review the circumstances of the work then accomplished, with such intimate details as are usually omitted from formal publications. You are therefore invited to embark upon a sentimental journey, less engaging than that of Lawrence Sterne through France and Italy, but somewhat more urologically oriented.

Our initial interest was in serum *alkaline* phosphatase and its relationship to bone formation in disorders affecting the skeleton, an interest inspired by the pioneer investigations in this field by Dr. Aaron Bodansky, my erstwhile instructor in physiological chemistry at the Ithaca branch of Cornell Medical College, and later a close neighbor at the Hospital for Joint Diseases. In the course of these earlier studies[1] we had found, as had Woodard, Twombly, and Coley,[2] that the serum alkaline phosphatase was usually substantially higher in the presence of widespread osteoplastic metastases, notably secondary to prostatic carcinoma, than in association with osteolytic metastases due, for example, to breast carcinoma. This increase in serum alkaline phosphatase was generally assumed to arise from augmented osteoblastic activity, with consequently increased elaboration of the enzyme. To put this hypothesis to the test we began in 1935 to examine the alkaline phosphatase activity of bone at the site of osteoplastic metastases sec-

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TABLE I—ACID AND ALKALINE PHOSPHATASE ACTIVITY OF OSTEOPLASTIC BONE METASTASES SECONDARY TO PROSTATE CARCINOMA COMPARED WITH NORMAL BONE AND OSTEOLYTIC METASTASES (BREAST CARCINOMA, MULTIPLE MYELOMA)*

Units/gm. fresh tissue in aqueous extract, phenylphosphate substrate

	Vertebra	
	Acid	*Alkaline*
Osteoplastic bone metastases, Ca prostate	19.0	39.4
Normal bone (man)	0.9	3.3
Osteolytic bone metastases, Ca breast	0.2	0.8
Multiple myeloma (bone)	1.3	2.2

*Data from Gutman, Sproul, and Gutman.[3]

ondary to prostatic carcinoma and were able to demonstrate that in such areas of vigorous new bone formation alkaline phosphatase was indeed present in abundance (Table I), much more than in normal cortical bone or in bone the site of osteolytic metastases arising from breast tumors.[3]

We were about to conclude this study when diverted by a most unexpected observation reported in 1935 by Kutscher and Wolbergs.[4] It seems that earlier in 1935 Dmochowski and Assenhajm,[5] while investigating the phosphatase activity of human urine, had noted in some subjects occasional sharp effluxes of urinary acid phosphatase, in amounts up to a 100-fold greater than the usual figures. This puzzling phenomenon was ascribed to episodic increased elimination of an acid phosphatase of renal origin. Sporadic outpourings of acid phosphatase in the urine were encountered also by Kutscher and Wolbergs, who at first thought they might be due to plant acid phosphatases derived from fresh fruits and vegetables taken in the diet,[6] but later noted that they occurred only in adult males, implicating the male genital tract as the source of the enzyme. This suspicion was confirmed when Kutscher and Wolbergs[4] discovered that the ejaculate contained an abundance of an acid phosphatase similar in properties to that of the enzyme in the urine. Proceeding to the autopsy table, they then found that the normal human prostate gland was extraordinarily rich in acid phosphatase; only minimal amounts were present in the testis, epididymis and seminal vesicles.[4]

We promptly confirmed these observations[3] (Table II) and extended them. It was shown that acid phosphatase was present in such abun-

TABLE II—ACID AND ALKALINE PHOSPHATASE ACTIVITY OF PROSTATE TISSUE, COMPARED WITH OTHER TISSUES, IN NORMAL ADULT MAN*

Units /gm. fresh tissue, phenylphosphate substrate

Case	Prostate		Liver		Kidney		Duodenum	
	Acid	Alkaline	Acid	Alkaline	Acid	Alkaline	Acid	Alkaline
1	997	0.4						
2	264	0.8						
3	605	0.5						
4	552	1.3	1.6	1.2	4.6	2.9	3.1	11.3
5	792	0.8	2.4	2.1	2.4	6.7	0.9	5.9
6	2284	0.9	2.6	10.6	3.1	2.3	0.8	4.0

*Data from Gutman, Sproul, and Gutman,[3] and Gutman and Gutman.[7]

TABLE III—SPECIES DIFFERENCES IN ACID PHOSPHATASE ACTIVITY OF ADULT PROSTATE TISSUE*

	Units/gm. fresh tissue
Man	525-2,300
Rhesus monkey (caudal lobe)	575-1,100
Dog	35
Guinea pig	3.9
Cat	2.8
Rat	2.0
Rabbit	1.9

*Data from Gutman and Gutman.[7]

dance only in the prostate of man and in the caudal lobe of the prostate gland of the Rhesus monkey; the prostate of the dog contained a much smaller amount, and the prostates of the cat, guinea pig, rabbit and rat contained very little[7] (Table III). In man and the Rhesus monkey the enzyme did not appear in significant quantity until sexual maturity[7, 8] (Tables IV and V), but its presence could be precociously induced in the immature Rhesus monkey by administration of testosterone;[8] estradiol had little or no effect (Table V). In regard to the high levels of acid phosphatase activity in human semen, it could be demonstrated that the prostate gland contributed its secretion to the seminal fluid early in the process of ejaculation—the first milliliter of the ejaculate is very rich in acid phosphatase and hence contains the bulk of the prostatic component (Table VI)—and, further, that while

TABLE IV—ACID PHOPHATASE ACTIVITY OF HUMAN PROSTATE TISSUE IN RELATION TO AGE*

Age	*Units/gm. fresh tissue*
Newborn	4.5
4 years	1.5
13 years	73.0
Adult	525-2,300

*Data from Gutman and Gutman.[7]

TABLE V—ACID AND ALKALINE PHOSPHATASE ACTIVITY OF PROSTATE TISSUE OF RHESUS MONKEY: SEXUALLY MATURE, IMMATURE, IMMATURE AFTER TESTOSTERONE PROPIONATE AND ESTRADIOL*

Status	*No. animals*	*Phosphatase activity (units/whole prostate)* Acid	Alkaline
Mature	3	2400	218
Immature	3	1.2	0.5
Immature given testosterone	2	1400	38
Immature given estradiol	1	7	1.5

*Data from Gutman and Gutman.[7]

there are marked differences in the acid phosphatase activity of the ejaculate of different individuals, the values are consistently of the same order of magnitude in any one person[9] (Table VII).

To return to the interrupted study of which I first spoke, that of bone at the site of osteoplastic metastases secondary to prostatic carcinoma, in view of Kutscher and Wolbergs' report[4] and our confirmation thereof, we hastened to measure the *acid* as well as the alkaline phosphatase activity of the bone metastases and found it too to be high, about 20 times greater than that of normal cortical bone, or of bone metastatically involved by tumors other than prostatic carcinoma, or of bone exhibiting increased alkaline phosphatase activity because of the presence of Paget's disease[3] (Table I). We were thus able to report in 1936 that carcinomatous prostate tissue, whether proliferating in distant metastases[3] or, as was soon determined,[10] at the primary site, does not lose the native capacity of normal human prostate tissue to secrete an acid phosphatase in abundance. This observation was itself of interest since it was contrary to the view then prevailing, namely, that malig-

TABLE VI—ACID PHOSPHATASE ACTIVITY OF FRACTIONATED EJACULATE IN NORMAL MAN*

Units/ml. fraction, phenylphosphate substrate

Fraction No.	*Volume (ml.)*	*Units acid phosphatase activity (per ml.)*
1	0.85	3250
2	1.40	830
3	0.85	350

*Data from Gutman and Gutman.[9]

TABLE VII—ACID PHOSPHATASE ACTIVITY OF SEMINAL FLUID IN NORMAL MAN*

Units/ml. seminal fluid, phenylphosphate substrate

Subject	*Specimen No.* 1	2	3	4	5	6
F	2450	2350	3060	2470	2710	2750
K	1960	1950	2320	2300	1630	2915
R	810	600	790	1040	710	805
B	3200	3350	3290	3170	3160	
G	1360	1370	1510	1920		
C	840	540	810	780		
H	3200	4040	4220			
S	3450	2250	3610			
A	2230	2020	2630			
W	1220	1410	960			

*Data from Gutman and Gutman.[9]

nancies derived from functionally immature, indeed embryonal cells.[11] Prostatic carcinoma evidently contained epithelial tissue physiologically mature enough to secrete acid phosphatase, a property not possessed by immature prostatic tissue.[7, 8] Of course some prostatic carcinomas are functionally as well as structurally undifferentiated, as in a case cited in 1939[12] in which the acid phosphatase activity of the primary tumor was found to be only 52 units per gram tissue, about 1/10th to 1/40th that of normal prostate. As Woodard has made clear, less pronounced reductions in enzyme are found in many prostatic carcinomas.[13]

Another interesting aspect of the presence of so much more acid phosphatase at the site of osteoplastic metastases secondary to prostatic

carcinoma than in any other disorder affecting the skeleton related to the old problem, why prostatic carcinoma, of all soft tissue tumors, is so much more likely to elicit the formation of predominantly *osteoplastic* bone metastases. In our initial 1936 report[3] we reviewed the various hypotheses that had been proposed to explain this phenomenon, concluding that the most generally accepted view at the time, derived from morphological studies of bone metastases, postulated the elaboration by certain tumor cells, such as prostatic cancer, of some unknown chemical factor that initiated the osteoplastic reaction. Was acid phosphatase this chemical factor in the case of metastatic prostatic carcinoma? Clearly, prostatic acid phosphatase itself did not cause bone formation directly (else prostate glands presumably would be completely ossified) but there was the question, which was left to future investigation, whether the enzyme might in some way indirectly stimulate the production of alkaline phosphatase by osteoblasts and thus the osteoplastic tendencies of skeletal metastases secondary to prostatic carcinoma.[3] Today we know that osseous acid phosphatase is an important component of the lysosomal apparatus of the osteoclast,[14] and that osteolysis may precede and stimulate osteoblastic bone reaction (as in hyperparathyroidism). Hence the question is still appropriate, but still unanswered.

Finally, and of particular relevance to the urologist, our finding of prostatic acid phosphatase at distant sites of metastatic proliferation of prostatic carcinoma raised the possibility of entry of the enzyme into the blood under these circumstances, with diagnostic implications. This question was investigated independently by Dr. Woodard and ourselves, both groups reporting in 1938.[10, 15] We recorded unequivocally increased serum acid phosphatase activity, measured at *p*H 4.9, in 11 of 15 cases of metastasizing prostatic carcinoma, due to the presence of an enzyme indistinguishable from prostatic acid phosphatase (Table VIII). In general, the highest values were found in cases with the most florid and extensive metastatic involvement, but there were exceptions. Only one of 88 patients with diseases other than disseminated prostatic carcinoma yielded serum values in excess of 4.0 acid phosphatase units, although many had marked elevations in serum alkaline phosphatase. It was concluded that the determination of serum acid phosphatase activity "may be of limited value in the diagnosis of metastasizing carcinoma of the prostate gland."[10] A similar conclusion

TABLE VIII—SERUM ACID AND ALKALINE PHOSPHATASE ACTIVITY IN 15 PATIENTS WITH PROSTATIC CARCINOMA*

Acid phenol units	*Alkaline Bodansky units*	*Skeletal metastases*
516	7.2	++++, osteolytic
40.8	12.9	+++, osteolytic-osteoplastic
38.6	17.3	++++, osteoplastic-osteolytic
26.3	21.4	++++, osteoplastic
10.7	4.2	+, osteolytic-osteoplastic
10.6	27.7	+++, osteolytic-osteoplastic
7.4	19.0	++, osteoplastic-osteolytic
6.8	9.5	++, osteoplastic
5.6	4.5	++, osteolytic-osteoplastic
5.4	50.0	++++, osteoplastic
4.4	21.6	+++, osteoplastic-osteolytic
3.1	20.1	+++, osteoplastic
1.6	2.8	±
1.6	12.0	+, osteolytic-osteoplastic
1.5	3.9	0

*Data from Gutman and Gutman.[10]

was reached by Barringer and Woodard,[15] whose measurements were made at *p*H 6.4 and because of overlap with serum alkaline phosphatase activity expressed the results as the ratio of acid to alkaline phosphatase values; despite these technical difficulties, in three of their 11 cases distinctly greater than normal ratios could be demonstrated.

We interpreted these observations as follows:[11] "When carcinomatous prostatic tissue metastasizes, invasion of lymph or blood channels is accompanied by escape of the prostatic secretion into the circulation; under these circumstances prostatic fluid becomes, so to speak, an internal as well as an external secretion. Because of its high acid phosphatase content, the prostatic secretion present in blood can readily be detected by means of appropriate chemical methods for estimating the acid phosphatase activity of blood serum, which is increased by influx of the prostatic enzyme."

At this juncture it seemed appropriate, if the determination of serum acid phosphatase activity were to be generally applicable to the diagnosis of metastasizing prostatic carcinoma, to define normal values and methodology more precisely. Roche[16] had examined the serum alkaline phosphatase activity over a wide range of *p*H, extending as low as 6.0, but measurements of phosphatase activity at lower *p*H could not be

TABLE IX—SERUM ACID PHOSPHATASE LEVELS IN NORMAL HUMAN ADULTS AND CHILDREN*

Units/100 ml. serum, phenylphosphate substrate

	Acid	*Alkaline*
Normal adult males (9)	1.2	11.6
Normal adult females (1)	1.0	6.8
Normal children (2)	1.9	38.0

*Data from Gutman and Gutman.[17]

found in the literature. We reported in 1938 that the sera of normal men, women, and children uniformly exhibit very low but detectable phosphatase activity (Table IX) optimal over an acid *p*H range of 4.0 to 6.5, due to the presence of an enzyme distinct from alkaline phosphatase, erythrocyte phosphatase and prostatic acid phosphatase.[17] The source of this serum acid phosphatase was obscure, but it was suggested that it might be liver or spleen, which were known to contain similar acid phosphatases. In 1958 Zucker and Borrelli found that, in fact, the bulk of the serum acid phosphatase in normal man derives from platelet acid phosphatase,[18] discovered in 1945 by Chevillard;[19] but there may well be other minor sources of what appear to be several isozymes present (see discussion by Mann[20]). Spurious increases in serum acid phosphatase activity doubtless arise most frequently in clinical practice as a result of hemolysis of red cells,[21] which must be avoided since erythrocytic phosphatase is relatively abundant and exhibits distinct although not optimum activity at *p*H 5.0.

For the measurements of serum acid phosphatase activity thus far mentioned we had, on the basis of preliminary trials, employed as substrate disodium monophenylphosphate, which had been introduced in 1934 by King and Armstrong for the estimation of serum alkaline phosphatase,[22] modifying the procedure to incubate with citrate buffer at *p*H 4.9. A more rigorous analysis of the conditions of the reaction was now made,[23] which indicated that these choices were about optimal for the purpose, so the details of the procedure were standardized for general use. Later, in 1950, we described a simplified and more rapid test for serum acid and alkaline phosphatase using phenolphthalein monophosphate as substrate.[24]

Of the large number of methods now available for estimating serum

TABLE X—PERCENTILE DISTRIBUTION OF SERUM ACID PHOSPHATASE IN PROSTATIC CARCINOMA AND OTHER DISEASES*

	No. of Cases	*Per cent of cases*					
		<3u	*3-5u*	*5-10u*	*10-20u*	*20-100u*	*>100u*
Normal	30	100					
Prostate Ca							
1. Metastatic (x-ray)	130	15	12	25	16	19	13
2. Nonmetastatic (x-ray)	70	89	11				
Benign prostatic hypertrophy	75	100					
Neoplasia other than prostatic							
1. Metastatic	145	90	6	4			
2. Nonmetastatic	64	94	5	1			
Primary bone tumors	31	90	10				
Paget's disease	96	79	18	3			
Hyperparathyroidism	9	67	11	22			
Miscellaneous diseases	225	97	1.5	0.5	1		

*Data from Sullivan, Gutman, and Gutman.[29]

acid phosphatase I shall mention only the refinement introduced in 1953 by Fishman and Lerner.[25] Using L-tartrate, as described by Abul-Fadl and King,[26] to inhibit the prostatic acid phosphatase component of the serum acid phosphatase activity, Fishman and his coworkers, in an extensive experience,[27] reported increased sensitivity and specificity of the method, notably in the detection of early spread of prostatic carcinoma. There is, however, some difference of opinion as to whether the inhibition of prostatic acid phosphatase by L-tartrate is entirely specific, and it is still uncertain whether the general clinical usefulness of the modification is appreciably greater than the determination of serum total acid phosphatase activity (see review by Woodard).[28]

With respect to the sensitivity and specificity of the serum (total) acid phosphatase activity in the detection of metastasizing prostatic carcinoma, with the help of Drs. John N. Robinson and Thomas J. Sullivan we undertook a more detailed appraisal than in our initial communication,[10] as did many others. A report issued in 1942 summarized our experience to that date, comprising 285 patients with diseases of the prostate gland, 30 normal subjects, and 570 patients with disorders other than of the prostate[29] (Table X). Of 130 patients with cancer of the prostate and roentgenologically demonstrated dissemination of tumor, 15 per cent gave serum acid phosphatase values

within our normal range (<3 units), 12 per cent gave equivocal values up to 5 units, and 73 per cent had diagnostic levels in excess of 5 units. In 70 patients with prostatic carcinoma but without radiographic evidence of metastasis, all had serum acid phosphatase activity less than 5 units, 89 per cent of them less than 3 units. Seventy-five cases of benign prostatic hypertrophy uniformly gave normal values. On the whole, the subsequent general experience appears to have been similar. According to a 1963 report by Schwartz, Greenberg, and O. Bodansky,[30] additional information concerning disseminated prostatic cancer can be procured by simultaneous determinations of serum phosphohexose isomerase and isocitric dehydrogenase.

To return to the 1942 summary of our experience,[29] of 209 cases of neoplasm other than prostatic, 145 of them with metastatic spread of the tumor, 5 per cent gave elevated serum acid phosphatase values, in the range of 5 to 10 units, as did 3 per cent of 96 cases of Paget's disease, 22 per cent of 9 cases of hyperparathyroidism, and 1.5 per cent of patients with miscellaneous disorders (Table X). No cases of Gaucher's disease were included in our control group, and we missed the modest but fairly consistent and diagnostically significant increase in that disorder recorded in 1956 by Tuchman, Suna, and Carr.[31]

A special study was made of the circumstances attending the overlap in Paget's disease[22] in the hope that the estimation of serum acid phosphatase activity would prove to be of aid in the occasionally difficult roentgenographic differentiation from osteoplastic bone metastases secondary to prostatic carcinoma; the serum alkaline phosphatase does not help in this situation as it characteristically is increased in both disorders. Despite the overlap this appeared to be the case since it was found that serum acid phosphatase levels above the upper limits of normal occurred in Paget's disease almost exclusively in patients with such widespread and typical skeletal involvement as hardly to be confused with osteoplastic bone metastases. In such instances the serum alkaline phosphatase usually was inordinately high, but it could be demonstrated, by inhibition with sodium fluoride and in other ways, that the enzyme activity at *p*H 4.9 was not due to residual scission by serum alkaline phosphatase.[32] Nor was the surplus serum acid phosphatase activity attributable to the presence of prostatic acid phosphatase (since it occurred also in some women with advanced Paget's disease) but, it was suggested,[11, 29] this might be due to the escape of small

quantities of bone acid phosphatase into the circulating fluids when there is extensive remodeling of bone.

The presence of an acid phosphatase in bone apparently had not been noted until it was encountered in our initial (1936) study of the acid phosphatase activity of aqueous extracts of normal and abnormal bone;[3] and even then the unavoidable admixture of erythrocytes in some preparations left the significance of these crude observations in doubt. Supporting evidence was procured, however, in observations on cases of hyperparathyroidism accompanied by an increase in serum acid phosphatase, since ablation of the parathyroid tumor (and with this, cessation of bone dissolution) was associated not only with prompt return of the serum calcium but also of the serum acid phosphatase to normal.[12, 29] The minor increases in serum acid phosphatase encountered in occasional cases of multiple myeloma, breast cancer, bronchogenic carcinoma, etc., extensively involving the skeleton also might be due to mobilization of bone acid phosphatase, likewise the modest rise in serum acid phosphatase observed in two of four cases of osteopetrosis, even though this condition is characterized by condensation of cortical bone ("marble bones").[29] Of course, the subsequent application of modern chemical and histochemical techniques to bone by later investigators has unequivocally demonstrated the presence of one or more acid phosphatases in bone.[14, 33, 34]

Such was the state of affairs when in 1941 Huggins and Hodges[35] published their classic paper demonstrating that the increased serum acid phosphatase of patients with disseminated prostatic carcinoma can be reduced promptly and sharply by orchiectomy or by injection of estrogens, with accompanying regression of the tumor and striking clinical improvement. These results were promptly confirmed by many investigators, including ourselves.[29] Thus the determination of serum acid phosphatase activity entered the current era, in which it is employed not only as an aid in the recognition of spread of prostatic carcinoma, but also as a guide in management. The facts are so familiar to this audience that I need not repeat them. It might be appropriate to point out, however, that the impact of the early investigations on the correlations between the serum alkaline and acid phosphatase levels and specific diseases of the skeleton, liver, and prostate probably extended beyond these organs in stimulating the currently burgeoning use of serum enzyme determinations in the diagnosis and manage-

ment of a variety of disorders. Although much has been accomplished, it is safe to say that, in view of the rapid progress in knowledge of enzymes, the surface has hardly been scratched.

Before concluding these reminiscences I should like to make some brief comments about the nonspecific acid phosphatases in general and prostatic acid phosphatase in particular. The designation, acid phosphatase, is a misnomer, since these enzymes are neither acid nor phosphatases. For a long time I put "acid" and "alkaline" in quotes, but it was a losing battle, and I finally surrendered when I received a characteristic note from Charlie Huggins to the following effect: For Heaven's sake, when are you going to stop putting quotes around acid and alkaline phosphatases! The nonspecific so-called phosphatases, acid and alkaline, are in fact hydrolases, cleaving the P-O bond with separation of the phosphoryl group from the orthophosphoric monoesters of a wide variety of phenolic, alcoholic, sugar and other compounds. The phosphoryl group may be transferred not only to water but also directly to a number of organic hydroxyl compounds; in this sense the enzymes act as phosphotransferases. The terms acid and alkaline phosphatases are so ingrained, however, that I doubt that prostatic acid phosphatase will ever be called by its imposing modern name, orthophosphoric monoester phosphohydrolase, EC 3.1.3.2!

There has been considerable recent progress in purification of prostate acid phosphatase, among others by London and Hudson,[36] Bowman,[37] and Ostrowski and Rybarska.[38] Boman and Ostrowski and Rybarska have obtained sufficiently homogeneous preparations on ultracentrifugal analysis to estimate the molecular weight at about 96,000. There is no information, so far as I am aware, of the amino acid composition or configuration of the molecule, or the nature of its active center or substrate-binding groups. Whether serine acts as a phosphate acceptor, as in the case of alkaline phosphatase, has not been established to my knowledge.

Nonspecific acid phosphatases are very widely distributed in nature, both in the plant and animal world, but the presence of so much acid phosphatase in the human prostate gland implies some special physiological role. The nature of this function, recently discussed by Mann[20] with special reference to phosphorylcholine (which is split by the enzyme) and choline[39] is, however, still unknown. It can be said that the enzyme probably does not act within the prostate gland (apart,

possibly, from contributing to the formation of corpora amylacea and calculi) but rather is destined for excretion in the seminal fluid. It seems unlikely also that prostate acid phosphatase has any profound and general significance in reproduction, in view of its very limited species distribution. In speculating on this point in 1942 we ventured to suggest that the enzyme might have something to do with the "metabolic processes providing energy required by human sperm for prolonged motility in inseminated ejaculates."[11] I am not aware of any convincing evidence in support of this or any other hypothesis.

REFERENCES

1. Gutman, A. B., Tyson, T. L. and Gutman, E. B. Serum calcium, inorganic phosphorus and phosphatase activity in hyperparathyoidism, Paget's disease, multiple myeloma and neoplastic disease of the bones. *Arch. Int. Med. 57:* 379-413, 1936.
2. Woodard, H. Q., Twombly, G. H. and Coley, B. L. A study of the serum phosphatase in bone disease. *J. Clin. Invest. 15:*193-201, 1936.
3. Gutman, E. B., Sproul, E. E. and Gutman, A. B. Significance of increased phosphatase activity of bone at the site of osteoplastic metastases secondary to carcinoma of the prostate gland. *Amer. J. Cancer 28:*485-95, 1936.
4. Kutscher, W. and Wolbergs, H. Prostataphosphatase. *Hoppe Seyler Z. Physiol. Chem. 236:*237-40, 1935.
5. Dmochowski, A. and Assenhajm, D. Über Harn- und Blutphosphatase. *Naturwissenschaften 23:*501, 1935.
6. Kutscher, W. and Wolbergs, H. Über Harnphosphatase. *Naturwissenschaften 23:*558-59, 1935.
7. Gutman, A. B. and Gutman, E. B. "Acid" phosphatase and functional activity of the prostate (man) and preputial glands (rat). *Proc. Soc. Exp. Biol. Med. 39:*529-32, 1938.
8. Gutman, A. B. and Gutman, E. B. Adult phosphatase levels in prepubertal Rhesus prostate tissue after testosterone propionate. *Proc. Soc. Exp. Biol. Med. 41:*227-81, 1939.
9. Gutman, A. B. and Gutman, E. B. Quantitative relations of a prostatic component (acid phosphatase) of human seminal fluid. *Endocrinology 28:* 115-18, 1941.
10. Gutman, A. B. and Gutman, E. B. An "acid" phosphatase occurring in the serum of patients with metastasizing carcinoma of the prostate gland. *J. Clin. Invest. 17:*473-78, 1938.
11. Gutman, A. B. Serum "acid" phosphatase in patients with carcinoma of the prostate gland: present status. *J.A.M.A. 120:*1112-16, 1942.
12. Robinson, J. N., Gutman, E. B. and Gutman, A. B. Clinical significance of increased serum "acid" phosphatase in patients with bone metastases secondary to prostatic carcinoma. *J. Urol. 42:* 602-18, 1939.
13. Woodard, H. Q. Factors leading to elevations in serum acid glycerophosphatase. *Cancer 5:*236-41, 1952.
14. McLean, F. C. and Urist, W. R. *Bone,* 2d ed. Chicago, Univ. Chicago Press, 1961.
15. Barringer, B. S. and Woodard, H. Q. Prostatic carcinoma with extensive intraprostatic calcification. With a discussion of the possible role of prostatic phosphatase. *Trans. Amer. Ass. Genito-Urin. Surg. 31:*363-69, 1938.
16. Roche, J. Blood-Phosphatases. *Biochem. J. 25:*1724-33, 1931.
17. Gutman, A. B. and Gutman, E. B. "Acid" phosphatase activity of the serum of normal human subjects. *Proc. Soc. Exp. Biol. Med. 38:*470-73, 1938.

18. Zucker, M. B. and Borrelli, J. Platelets as a source of serum acid nitrophenylphosphatase, *J. Clin. Invest. 38*:148-54, 1959.
19. Chevillard, L. Sur les phosphomonestérases de la moelle du fémur, des ganglions lymphatiques, des leucocytes d'exsudats chez le cobaye, et des plaquettes sanguines chez le lapin, *C. R. Soc. Biol. (Paris) 139*:249-50, 1945.
20. Mann, T. *The Biochemistry of Semen and of the Male Reproductive Tract.* New York, Wiley & Sons, 1964.
21. Gutman, E. B. and Gutman, A. B. Erythrocyte phosphatase activity in hemolysed sera and estimation of serum "acid" phosphatases, *Proc. Soc. Exp. Biol. Med. 47*:513-15, 1941.
22. King, E. J. and Armstrong, A.R. A convenient method for determining serum and bile phosphatase activity, *Canad. Med. Ass. J. 31*:376-81, 1934.
23. Gutman, E. B. and Gutman, A. B. Estimation of "acid" phosphatase activity of blood serum, *J. Biol. Chem. 136*:201-09, 1940.
24. Gutman, A. B. Simplified serum alkaline and acid phosphatase method using phenolphthalein monophosphate substrate, *Fed. Proc. 9*:180, 1950.
25. Fishman, W. H. and Lerner, F. A method for estimating serum acid phosphatase of prostatic origin, *J. Biol.* Chem. *200*:89-97, 1953.
26. Abul-Fadl, M. A. M. and King, E. J. Properties of the acid phosphatases of erythrocytes and of the human prostate gland, *Biochem. J. 45*:51-60, 1949.
27. Cook, W. B., Fishman, W. H. and Clarke, B. G. Serum acid phosphatase of prostatic origin in the diagnosis of prostatic cancer: Clinical evaluation of 2,408 tests by the Fishman-Lerner method, *J. Urol. 88*:281-87, 1962.
28. Woodard, H. Q. The clinical significance of serum acid phosphatase, *Amer. J. Med. 27*:902-10, 1959.
29. Sullivan, T. J., Gutman, E. B. and Gutman, A. B. Theory and application of the serum "acid" phosphatase determination in metastasizing prostatic carcinoma; early effects of castration, *J. Urol. 48*:426-58, 1942.
30. Schwartz, M. K., Greenberg, E. and Bodansky, O. Comparative values of phosphatases and other serum enzymes in following patients with prostatic carcinoma: Consideration of phosphohexose isomerase, glutamic oxalacetic transaminase, isocitric dehydrogenase, and acid and alkaline phosphatases, *Cancer 16*:583-94, 1963.
31. Tuchman, L. R., Suna, H. and Carr, J. J. Elevation of serum acid phosphatase in Gaucher's disease, *J. Mount Sinai Hosp. 23*:227-29, 1956.
32. Gutman, A. B., Gutman, E. B. and Robinson, J. N. Determination of serum "acid" phosphatase activity in differentiating skeletal metastases secondary to prostatic carcinoma from Paget's disease of bone, *Amer. J. Cancer 38*:103-08, 1940.
33. Vaes, G. and Jacques, P. Studies on bone enzymes. The assay of acid hydrolases and other enzymes in bone tissue, *Biochem. J. 97*:380-88, 1965.
34. Vaes, G. and Jacques, P. Studies on bone enzymes. Distribution of acid hydrolases, alkaline phenylphosphatase, cytochrome oxidase and catalase in subcellular fraction of bone tissue homogenates, *Biochem. J. 97*:389-92, 1965.
35. Huggins, C. and Hodges, C. V. Studies on prostatic cancer. 1. The effect of castration, of estrogen and of androgen injection on serum phosphatases in metastatic carcinoma of the prostate, *Cancer Res. 1*:293-97. 1941.
36. London, M., Sommer, A. and Hudson, P. B. Further studies on the purification of prostatic acid phosphatase, *J. Biol. Chem. 216*:81-95, 1955.
37. Boman, H. G. Purification of prostatic acid phosphatase, *Arkiv Kemi 12*:453-65, 1958.
38. Ostrowski, W. and Rybarska, J. Studies on human prostatic acid phosphomonoesterase. Further purification and molecular weight of the enzyme, *Biochim. Biophys. Acta 105*:196-98, 1965.
39. Lundquist, F. Function of prostatic phosphatase, *Nature (London) 158*:710-11, 1946.